

Introduction

LGR5-expressing secretory cells regulate cartilage formation by inhibiting Wnt signaling during synovial joint development, guiding the spatial distribution of chondrocytes¹. While this regulatory role has been characterized in the appendicular skeleton, its function in other cartilaginous tissues, such as the intervertebral disk (IVD), remains unknown. The IVD is essential for flexibility and load distribution and develops from the notochord, which later forms the nucleus pulposus at the IVD's core. Understanding the mechanisms controlling IVD development and maintenance is critical, as degeneration of this tissue is a common cause of back pain and disability. If LGR5-expressing cells influence IVD formation and homeostasis, they may represent a key regulatory component and a therapeutic target for regenerative treatments aimed at restoring disk function and preventing degeneration.

Aims

- Identify LGR5 in the vertebral column
- Investigate overlap of known genes involved in intervertebral disk development and LGR5

Methods: Cryosectioning



Fig. 1. Tissue Sectioning. Slides were cut at 35 microns, which provides a compromise between maintaining tissue integrity and minimizing detachment from slides. Image produced in Biorender.

Methods: Immunohistochemistry

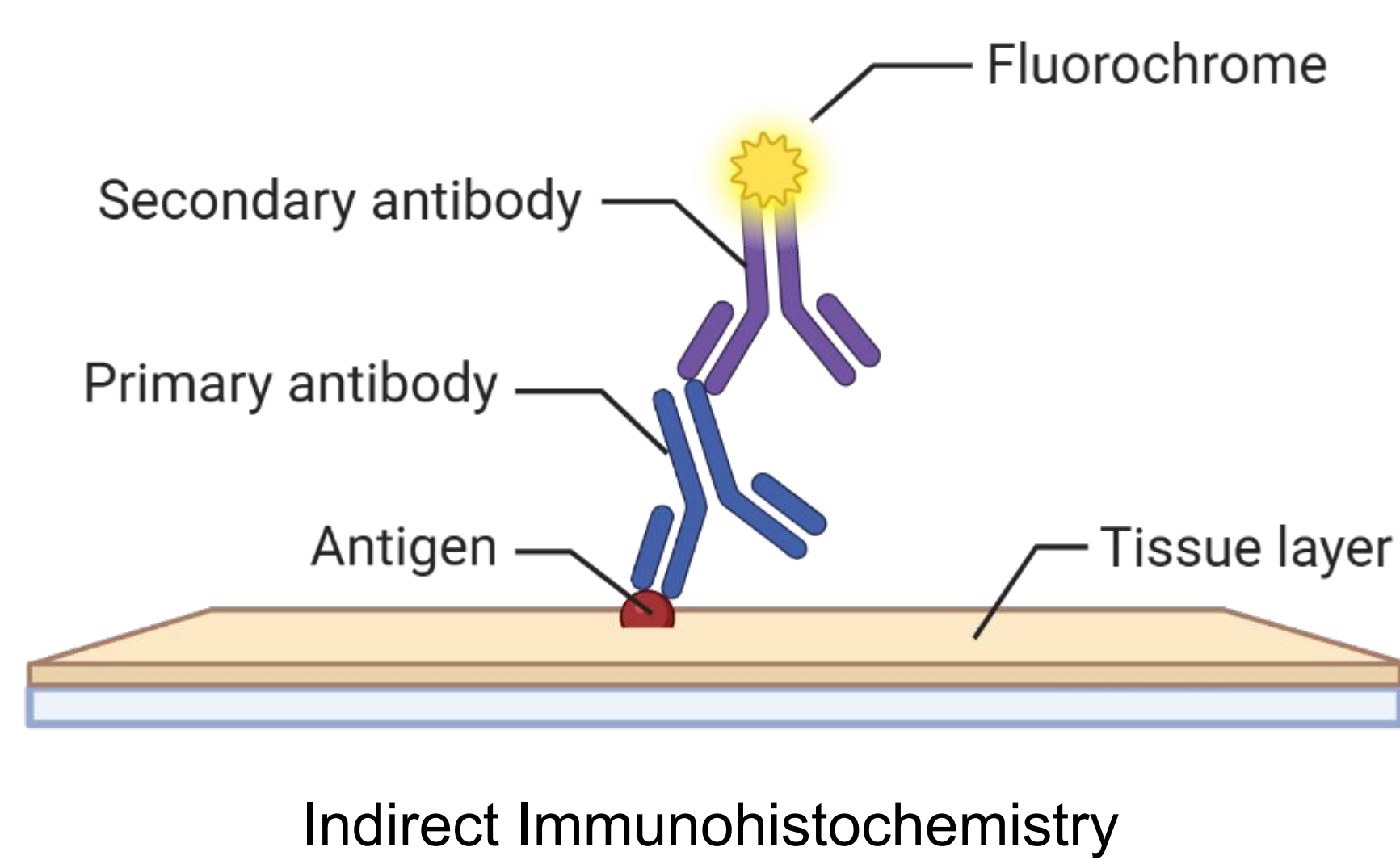


Fig. 2. Immunofluorescent detection of H2B-GFP. This study utilizes a gene-edited porcine model with LGR5 tagged with H2B-GFP, enabling fluorescent identification of LGR5⁺ cells during vertebral column development. Immunostaining was performed for TBXT (notochord landmark), and SOX10 (neural crest and IVD marker). This approach allows spatial visualization of LGR5⁺ cells and their role in IVD patterning and homeostasis. Image from Biorender.

Results

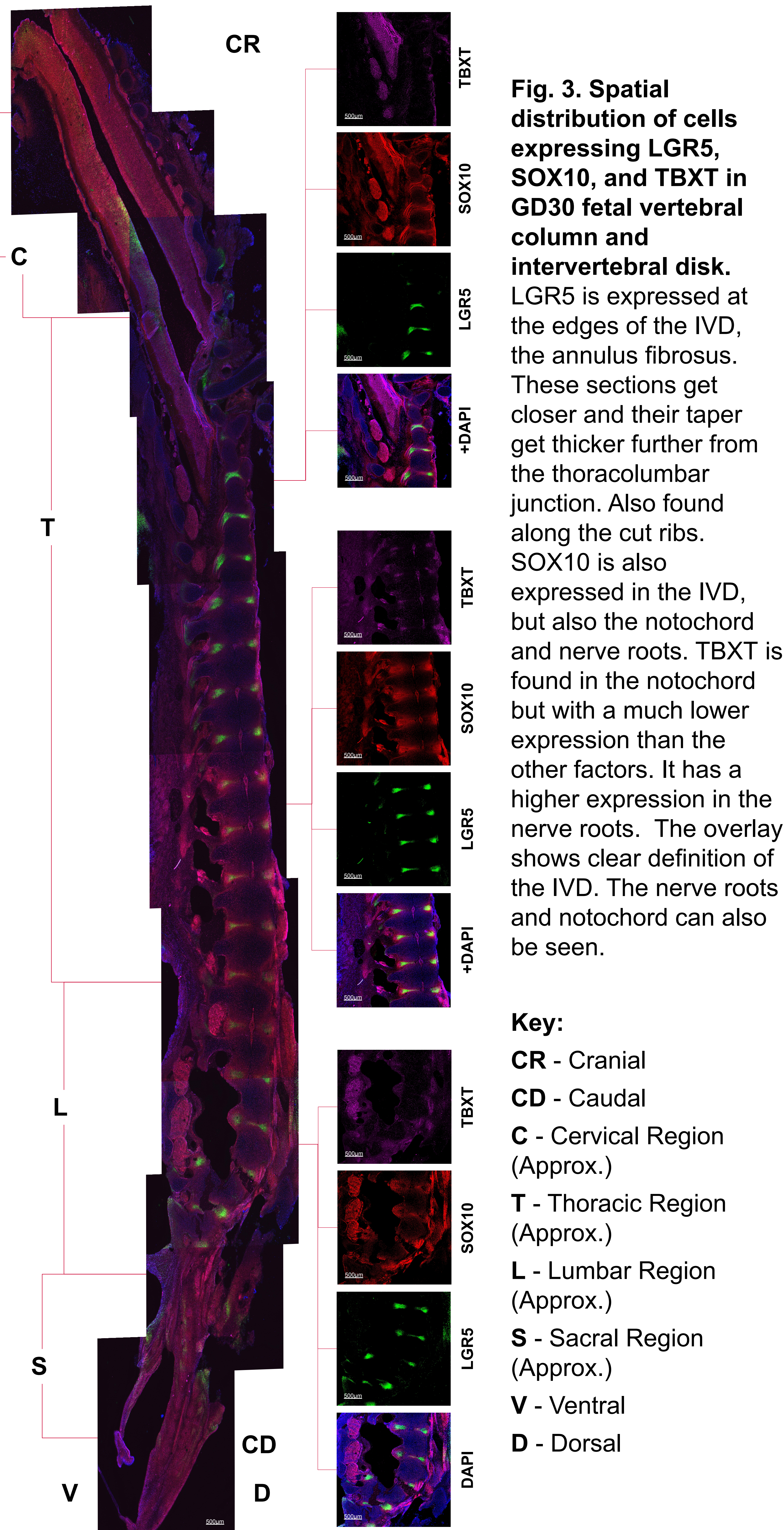


Fig. 3. Spatial distribution of cells expressing LGR5, SOX10, and TBXT in GD30 fetal vertebral column and intervertebral disk. LGR5 is expressed at the edges of the IVD, the annulus fibrosus. These sections get closer and their taper get thicker further from the thoracolumbar junction. Also found along the cut ribs. SOX10 is also expressed in the IVD, but also the notochord and nerve roots. TBXT is found in the notochord but with a much lower expression than the other factors. It has a higher expression in the nerve roots. The overlay shows clear definition of the IVD. The nerve roots and notochord can also be seen.

- Key:**
- CR - Cranial
 - CD - Caudal
 - C - Cervical Region (Approx.)
 - T - Thoracic Region (Approx.)
 - L - Lumbar Region (Approx.)
 - S - Sacral Region (Approx.)
 - V - Ventral
 - D - Dorsal

Anatomy of the Vertebral Column

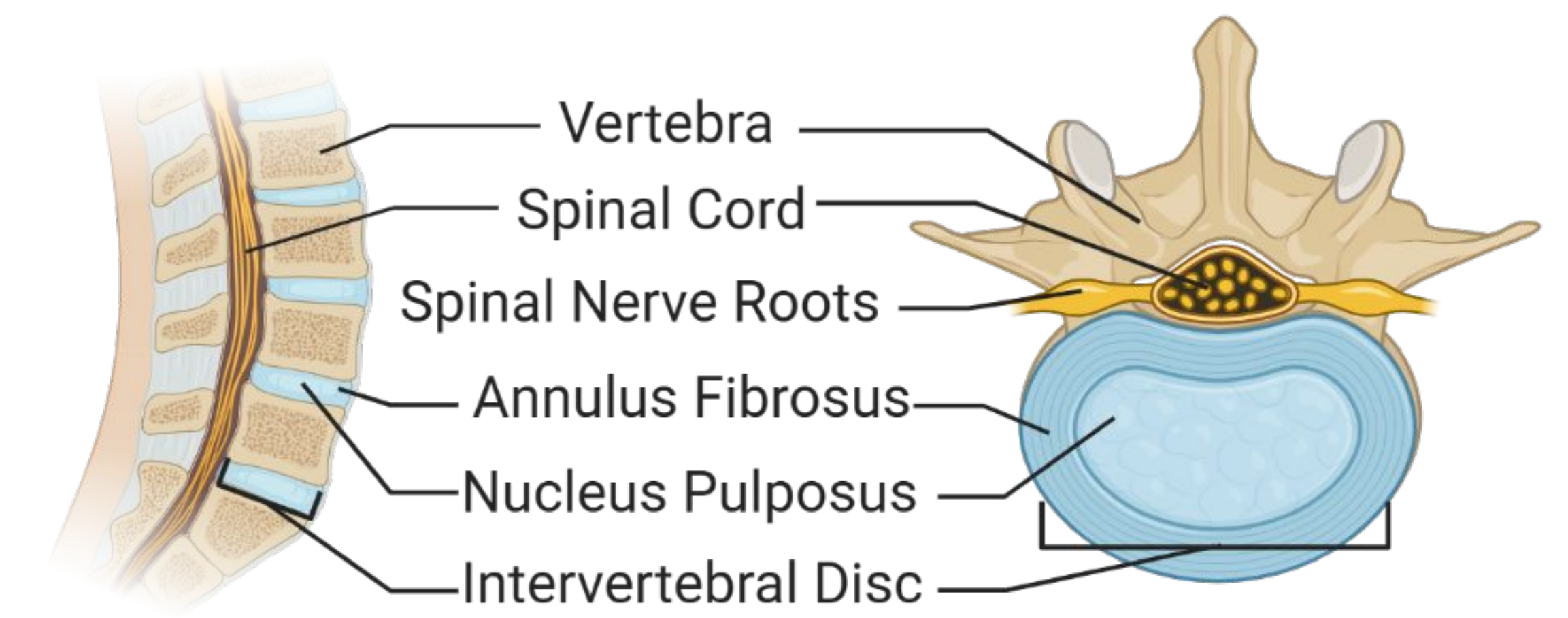


Fig. 4. Vertebral Column Diagram. Image produced in Biorender.

Overlap of LGR5 with SOX10 and TBXT

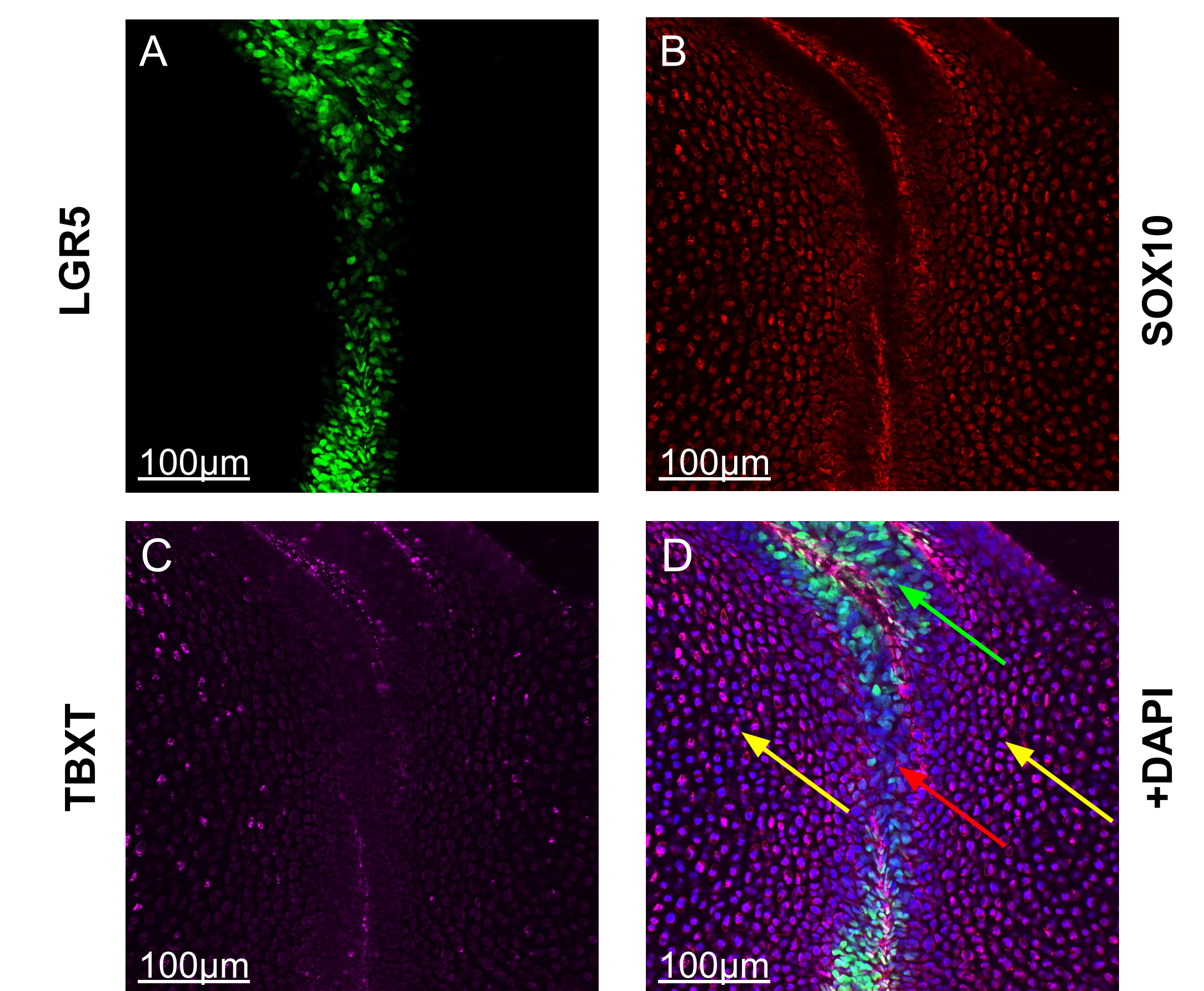


Fig. 5. Are LGR5, SOX10 and TBXT co-expressed? Co-expression is seen in only a few cells. But, there are high expressions of each in the annulus fibrosus. SOX10, a nuclear-localized protein, and TBXT, a cytoplasmic protein, were chosen for their abundance in the notochord, an area which does not express LGR5 as previously hypothesized. Additionally, there is a gradient of LGR5. This can be an effect of either the shape of the structure or the expression of the cells. It remains to be proven which case this is. **Green arrow** - annulus fibrosus, **red arrow** - nucleus pulposus, **yellow arrow** - vertebra.

Conclusions

Both SOX10 and TBXT have very low levels of co-expression with LGR5 expressing cells. However, they are found in higher expression groups near each other. This may still prove useful to study of the developing Vertebral column and IVD. There is a deeper relationship with these three factors that must be explored in future work. That work will be done with 3D imaging so that the shape of LGR5 expression can be fully understood.

References

1. Ruscitto, A., Chen, P., Tosa, I., Wang, Z., Zhou, G., Safina, I., Wei, R., Morel, M. M., Koch, A., Forman, M., Reeve, G., Lechlopol, M. K., Wilson, M., Bonthius, D., Chen, M., Ono, M., Wang, T. C., Yao, H., & Embree, M. C. (2023). *Lgr5-expressing secretory cells form a Wnt inhibitory niche in cartilage critical for chondrocyte identity.* *Cell Stem Cell*, 30(9), 1179–1198.e7.